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CROP PROTECTION

Mixture compatibility of *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) with pesticides used in soybean

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ABSTRACT: *Anticarsia gemmatalis (Hübner: 1818) (Lepidoptera: Erebidae) is one of the main pests that affect soybean crops, causing defoliation. In the vegetative stages, defoliation occurs together with weeds, and in the reproductive stages with pathogens. In this sense, to maintain plant health, it is necessary to carry out the combined use of pesticides. Thus, this research determined the compatibility of the entomopathogenic virus AgMNPV with the main herbicides and fungicides used in soy at different times of the mixture. The artificial diet was immersed in the solutions of the pesticides and their mixtures and supplied to A. gemmatalis caterpillars, immediately and after one and two hours of mixing. The evaluation was performed by quantifying the number of dead caterpillars by mixing the AgMNPV virus with herbicides and fungicides, even after two hours of mixing if compatible. The observed scenarios showed a compatibility of the virus with the herbicides and fungicides, with mortality rates between 70 to 99% for A. gemmatalis.*
Key words: *velvetbean caterpillar, baculovirus, tank mix, integrated pest management, biological control.*

Compatibilidade da mistura de nucleopoliedrovírus de *Anticarsia gemmatalis* baculovírus (AgMNPV) com agrotóxicos utilizados na soja

RESUMO: *Anticarsia gemmatalis (Hübner: 1818) (Lepidoptera: Erebidae) é uma das principais pragas que acometem a cultura da soja, causando desfolha. Nos estágios vegetativos a desfolha ocorre juntamente com ervas daninhas, e no reprodutivo com patógenos. Nesse sentido, para manter a fitossanidade, é necessário realizar a utilização combinada de pesticidas. Assim, o objetivo do presente trabalho foi determinar a compatibilidade do vírus entomopatogênico AgMNPV com os principais herbicidas e fungicidas utilizados na soja em diferentes tempos de mistura. A dieta artificial foi imersa nas soluções dos pesticidas e suas misturas e fornecida às lagartas de A. gemmatalis, imediatamente e após uma e duas horas de mistura. A avaliação foi realizada quantificando o número de lagartas mortas. A mistura do vírus AgMNPV com herbicidas e fungicidas, mesmo após duas horas de mistura se mostrou compatível. Os cenários observados mostram a compatibilidade do vírus com os herbicidas e fungicidas, com percentuais de mortalidade entre 70 a 99% para A. gemmatalis.*
Palavras-chave: *lagarta-da-soja, baculovírus, mistura de tanque, manejo integrado de pragas, controle biológico.*

INTRODUCTION

Soybean (*Glycine max* L. Merril) can be considered one of today's most important crops. It is responsible for most of the global demand for oil and vegetable protein (OERKE & DEHNE, 2004). From Argentina to the Southeast United States the velvetbean caterpillar *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Eribidae) has been one of its most important pest (BORTOLOTTO et al., 2015; PANIZZI & CORREA-FERREIRA, 1997). Despite the high efficacy of Bt soybean cultivars in controlling this pest, *A. gemmatalis* still is the most abundant species at early crop season (vegetative stage) in different important soybean areas in Brazil (CONTE et al., 2019). It is because non-Bt soybean was still cropped in 13.7 million of hectares during the 2017/2018 Brazilian crop season. Moreover, even at the maximum of Bt soybean adoption in Brazil, 7.2 millions of hectares of non-Bt soybean are still expected (COUNCIL BIOTECHNOLOGY INFORMATION, 2018), keeping pest management in non-Bt fields an important issue.

Anticarsia gemmatalis control is carried out, predominantly, with the use of synthetic insecticides (PLATA-RUEDA et al., 2020; STACKE et al., 2020). However, its overuse triggers impactful negative effects (BUENO et al., 2020; SONG &

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SWINTON, 2009), among which the selection of resistant pest strains (DIEZ-RODRÍGUEZ & OMOTO, 2001; KOCH et al., 2018) and the death of biological control agents (CARMO et al., 2010; HATT et al., 2018; VAN LENTEREN & BUENO, 2003) are the most relevant. Thus, a better alternative to the exclusive use of chemical pesticides in caterpillar pest management is the adoption of Integrated Pest Management (IPM). Not only can IPM offer a more profitable soybean yield due to the reduction of pest control costs but also assures equitable, secure, sufficient, and stable flows of yield and ecosystem services throughout the use of less harmful pest control strategies (BUENO et al., 2020).

The major IPM principle is to control pests throughout different tools combined in a compatible strategy. Moreover, environmentally safe management tool should be prioritized (BUENO et al., 2011; TORRES & BUENO, 2018). Among the most environment-friendly and sustainable pest management tools available, biological control stands out (VAN LENTEREN et al., 2018) in which the *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) is the world's most successful viral bioinsecticide (MOSCARDI, 1999; DEL-ANGEL et al., 2018). Considering that *A. gemmatalis* often occurs in soybean crops at the same periods of important weeds and plant pathogens (HARTMAN et al., 2011) it is of theoretical and practical interest to study the mixture compatibility of AgMNPV with different herbicides and fungicides. This study will allow knowing which herbicide or fungicide can be mixed with AgMNPV in the same spray's operation. The use of combined pesticides in a single spray is a desired practice in the management of soybean fields due to less soil compaction, decreased machinery's cost, and increased safety to growers due to reduced time spent spraying pesticides in the field (GANDINI et al., 2020; HENRY et al., 2011; PETTER et al., 2012). Not only is it important to evaluate the efficacy of pesticides immediately after the mixture but also at different time after products mixture since in field conditions, it might take hours for growers to spray the whole crop after preparing the sprayer with pesticides. Therefore, this research verified the mixture compatibility of AgMNPV with herbicides and fungicides commonly used in soybean crops both immediately and one and two hours after the mixture of those pesticides. This was carried out in order to point out which products might and might not be mixed in the field, considering possible effects of time after the mixture into the product`s compatibility.

MATERIALS AND METHODS

Eight independent bioassays were carried out evaluating *A. gemmatalis* mortality caused by herbicides or fungicides alone (bioassays 1 and 2), AgMNPV mixed with herbicides and fungicides immediately (bioassays 3 and 4) and both one (bioassays 5 and 6) and two hours (bioassays 7 and 8) after the mixture (Table 1). The bioassays were carried out under controlled conditions of temperature (25 ± 2°C), relative humidity (70 ± 10%) and photoperiod (14:10 h Light/Dark) at the Laboratory of Entomology from Embrapa Soja, located in Londrina, Paraná, Brazil.

Laboratory rearing of A. gemmatalis

Anticarsia gemmatalis caterpillars, used in the bioassays, originated from insect colonies kept at Embrapa Soybean (one of the units of the Brazilian Agricultural Research Corporation), Londrina, State of Paraná, Brazil. Colony was kept under controlled environmental conditions inside Biochemical Oxygen Demand (BOD) climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set at 70 ± 10% humidity, temperature of 25 ± 2°C, and a 14:10 h (L:D) photoperiod according to methodologies previously described in literature (SILVA et al., 2012) and briefly summarized in the followings.

Anticarsia gemmatalis were originally collected in soybean fields in Embrapa Soybean Experimental Farm, Londrina, State of Paraná, Brazil (23° 11' 11.7" S and 51° 10' 46.1" W). These populations were kept in the laboratory for approximately 3 yr during which new field insects were introduced each year to maintain colony quality.

Caterpillars were individually kept in 50-mL plastic cups (Plasvale Ltda., Gaspar, State of Santa Catarina, Brazil) sealed with cardboard caps, containing an artificial diet developed by GREENE et al. (1976). Adults were placed in cages (PVC tubes of 10 cm in diameter x 21.5 cm high) and fed with solution (10 g of honey, 60 g of sugar, 1 g of sorbic acid, 1 g of methylparaben / 1 liter of distilled water) and beer. Cages walls were covered with A4 paper for moth oviposition. Eggs were removed and cages cleaned daily. Eggs were placed into 200-mL plastic cups (Plasvale Ltda., Gaspar, State of Santa Catarina, Brazil) containing 20 mL of artificial diet (GREENE et al., 1976). The caterpillars were kept in these containers until they reached the 3rd instar when they were then used for trials.

Different pesticides alone (bioassay 1 and 2) and AgMNPV mixture with different herbicides and fungicides (bioassay 3 and 4) mortality of *Anticarsia gemmatalis.*

Table 1 - Herbicides and fungicides evaluated for mixture compatibility with AgMNPV (Baculovirus Soja WP® 20 g.150 L H_2O^{-1}) under controlled laboratory conditions (temperature of 25 ± 2°C, relative humidity of 70 ± 10% and photoperiod of 14:10 h Light/Dark) and commercial dosage.

Pesticide	Ative ingredient (a.i.)	Commercial product	Chemical group	Concentration of a.i.	Dosage (ha) p.c.
Bioassays 1, 3, 5 e 7					
Herbicides	clethodim	Poquer®	Cyclohexanedione	240 g/L	0.45 L.200 L H_2O^{-1}
	chlorimuron-ethyl	Clorimuron Master Nortox®	Sulfonylurea	250 g/kg	80 g.200 L H_2O^{-1}
	flumioxazin	Flumyzin 500®	Dicarboximide	500 g/kg	200 g.200 L H_2O^{-1}
	imazapic + imazapir	Soyvance Pre®	Imidazolinone + imidazolinone	525 g/kg + 175 g/kg	100 g.200 L H_2O^{-1}
	imazapic + imazapir	Soyvance®	Imidazolinone + imidazolinone	175 g/kg + 525 g/kg	100 g.200 L H_2O^{-1}
Bioassays 2, 4, 6 e 8					
Herbicides	bentazone	Basagran 600®	benzothiadiazinone	600 g/L	1.6 L.200 L H_2O^{-1}
	clethodim	Select 240 EC®	Cyclohexanedione	240 g/L	0.45 L.200 L H_2O^{-1}
	Glyphosate-isopropylamine salt	Trop®	Organophosphorus	480 g/L	2 L.200 L H_2O^{-1}
Fungicides	azoxystrobin + benzovindiflupyr	Elatus®	Strobilurin + pyrazolcarboxamide	300 g/kg + 150 g/kg	300 g.200 L H_2O^{-1}
	azoxystrobin + cyproconazole	Priori Xtra®	Strobilurin + triazole	200 g/L + 80 g/L	0.3 L.200 L H_2O^{-1}
	cyproconazole + trifloxystrobin	Sphere Max®	Strobilurin + triazole	160 g/L + 375 g/L	0.2 L.200 L H_2O^{-1}
	metconazole + pyraclostrobin	Opera Ultra®	Strobilurin + triazole	80 g/L + 130 g/L	0.6 L.200 L H_2O^{-1}

Different herbicides and fungicides (Table 1) used alone or in association with the entomopathogenic baculovirus AgMNPV (Baculovirus Soja WP® 20g.150 L H_2O^{-1}) were evaluated in the dosage recommended by the manufacturers. As a control, sterilized distilled water was used. Artificial diet (GREENE et al. 1976) was cut into cubes (1 cm^3) and immersed in the treatments for 5 seconds, then placed in plastic containers with a volume of 50 mL (sample unit), containing 2 caterpillars of 3rd instar. The diet cubes with the treatments were offered for caterpillars for 24 hours and later exchanged for cubes of a diet free of any pesticide.

The bioassay was conducted in a completely randomized design. Each treatment consisted of four repetitions containing 20 caterpillars each, which were packed in pairs in the sample units, totaling 80 caterpillars per treatment. The evaluation was performed daily, quantifying the number of dead caterpillars. The specimen was considered dead when it was immobile and insensitive to mechanical touch using a forceps.

Anticarsia gemmatalis mortality due to AgMNPV in mixture with different herbicides and fungicides after one (bioassay 5 and 6) and two (bioassay 7 and 8) hours of the mixture.

Treatments formed by the products (Table 1) and their association with AgMNPV (Baculovirus Soja WP® 20 g.150 L H_2O^{-1}) were evaluated in a completely randomized design. As previously described for bioassays 1, 2, 3 and 4 each treatment consisted of four repetitions containing 20 caterpillars each, which were packed in pairs in the sample units, totaling 80 caterpillars per treatment. The evaluation was performed daily, quantifying the number of dead caterpillars. The specimen was considered dead when it was immobile and insensitive to mechanical touch using a forceps.

Statistical analysis

The data obtained in the bioassays were analyzed for normality (SHAPIRO & WILK, 1965) and homogeneity of variance for each treatment (BURR & FOSTER, 1972) and, if necessary, transformed to perform ANOVA. The treatment means were then compared by Tukey test at the 5% probability level (SAS, 2001).

RESULTS

The caterpillars treated only with herbicides and fungicides, without association with

AgMNPV (bioassays 1 and 2) triggered similar mortality (%) to control without the virus (8.74% and 10.23%, respectively). Those results were lower than mortality caused by AgMNPV (98.62% and 97.75%, respectively). Only the fungicides metconazole + pyraclostrobin caused a higher mortality of *A. gemmatalis* (40.95%) compared to the respective control (10.23%) (Table 2).

Regarding herbicides and fungicides, when associated with AgMNPV (bioassays 3 and 4), they did not differ from the mortality (%) caused by the AgMNPV alone (95.22% and 93.45%, respectively), where the mortality rates ranged from 89.29% (clethodim) to 99.31% (chlorimuron-ethyl). Low mortality rates were observed in controls without AgMNPV (6.80% and 2.89%, respectively) (Table 2).

When evaluating the mixture compatibility of AgMNPV with herbicides and fungicides one hour after mixing the products (bioassays 5 and 7), it was reported that the herbicides and fungicides did not reduce the mortality of *A. gemmatalis* compared to AgMNPV alone (95.76% and 80.11%, respectively). Mortality ranged from 80.60% (azoxystrobin + benzovindiflupyr) to 99.31% (imazapic + imazapir). The mortality values caused by the pesticides were all higher than the values registered for the respective controls (2.83% and 6.67%) (Table 3).

Likewise, when checking the AgMNPV mixture compatibility with herbicides and fungicides two hours after mixing (bioassays 6 and 8), *A. gemmatalis* mortality caused by all the tested pesticides were higher than the mortality caused by the respective controls (1.36% and 2.00%). Although, azoxystrobin + benzovindiflupyr mixed with AgMNPV caused lower mortality of the caterpillars (69.27%) compared to the herbicide Bentazone

Table 2 - *Anticarsia gemmatalis* mortality (%) due to ingestion of different pesticides alone (bioassays 1 and 2) or in mixture (bioassay 3 and 4) with AgMNPV (Baculovirus Soja WP® 20 g.150 L H_2O^{-1}).

Pesticides (with and without mixing with *Ag*MNPV)		(Without mixture with AgMNPV) (bioassay 1)	(When mixed with AgMNPV) (bioassay 3)
control with virus (*Ag*MNPV)		98.62±0.84a	95.22±2.06a
Herbicides	Clethodim (Poquer)	8.14±1.70b	97.33±1.63a
	Chlorimuron-ethyl	7.38±1.24b	99.31±0.69a
	Flumioxazin	7.2±2.52b	98.57±1.43a
	Imazapic + imazapir (Soyvance Pré)	10.83±1.29b	97.88±1.42a
	Imazapic + imazapir (Soyvance)	9.64±1.39b	97.20±1.33a
Control without virus (Distilled water)		8.74±1.71b	6.80±1.49b
CV (%)		16.63	3.92
F		450.05	534.77
p		<0.0001	<0.0001
$df_{residue}$		28	28
Pesticides (with and without mixing with *Ag*MNPV)		(Without mixture with *Ag*MNPV) (bioassay 2)	(When mixed with *Ag*MNPV) (bioassay 4)
Control with virus (AgMNPV)		97.75±1.49a	93.45±1.50a
Herbicides	Bentazone	6.09±1.29c	95.28±2.25a
	Clethodim (Select)	6.67±1.49c	89.29±3.22a
	Glyphosate-isopropylamine salt	10.67±1.94c	95.89±1.26a
Fungicides	Azoxystrobin + cyproconazole	13.74±3.66c	95.88±3.34a
	Azoxystrobin + benzovindiflupyr	0.70±0.69d	97.31±1.95a
	Metaconazole + pyraclostrobin	40.95±6.45b	97.71±0.94a
	Trifloxystrobin + cyproconazole	9.50±1.94c	97.24±2.01a
Control without vírus (Distilled water)		10.23±1.90c	2.89±1.38b
CV (%)		22.01	5.62
F		92.24	209.23
p		<0.0001	<0.0001
$df_{residue}$		36	36

Means ± EPM followed by the same letter in each column do not differ by Tukey's test (5% probability).

(97.93%), both led to mortalities similar to that caused by the respective control (84.89%), as well as the other evaluated pesticides, when compared to the respective controls (96.40% and 84.89%) (Table 3).

DISCUSSION

The efficiency of the entomopathogenic virus AgMNPV has been proven since the 1980s (MOSCARDI, 1999) to the present date, with high levels of control even after three decades (DEL-ANGEL et al., 2018). However, soybean production in this period increased exponentially, from 15 million tonnes in the 1980s to 114 million in 2017 (CATTELAN & DALL'AGNOL, 2018), this significant increase was also due to the increase of cropped area that was followed by higher incidence of weeds and plant pathogens.

Mixing different pesticides (insecticides with herbicides or fungicides) was started as an alternative to reduce the costs of the increased need to control both insects and weeds or plant pathogens ate the same time (GANDINI et al., 2020; ZANDONADI et al., 2018). However, the mixture of different molecules can reduce control of these substances. Studies verifying compatibility between molecules are essential to assess the feasibility of those pesticide mixture with the same quality as the isolated use. Studies evaluating the compatibility of synthetic chemical insecticides and herbicides were carried out, which verified that most of the molecules are compatible, demonstrating the efficiency of the mixture of substances in pest control (GANDINI et al., 2020; MA et al., 2016; PETTER et al., 2012); however, as far as we know this is the first research evaluating the mixture compatibility of chemical pesticides with AgMNPV.

In the present study, the entomopathogenic virus AgMNPV was compatible with tested fungicides

Table 3 - *Anticarsia gemmatalis* mortality (%) due to ingestion of AgMNPV (Baculovirus Soja WP® 20 g.150 L H_2O^{-1}) in mixture with different pesticides after 1 hour (bioassays 5 and 6) and two hours (bioassays 7 and 8) of the mixture.

Pesticides		-----------------Time after the mixture with AgMNPV--------------	
		1 hour (bioassay 5)	2 hours (bioassay 7)
Control with virus (AgMNPV)		95.76±1.34a	96.40±2.03a
Herb	Clethodim (Pôquer)	93.65±2.91a	96.50±1.13a
	Chlorimuron-ethyl	95.93±1.63a	97.93±0.84a
	Flumioxazin	97.78±2.22a	99.23±0.77a
	Imazapic + imazapir (Soyvance pré)	99.31±0.69a	98.62±0.84a
	Imazapic + imazapir(Soyvance)	99.31±0.68a	98.00±2.00a
Control without virus (Distilled water)		2.83±2.08b	1.36±0.83b
CV (%)		10.37	9.00
F		77.50	107.15
P		<0.0001	<0.0001
$df_{residue}$		28	28
Pesticides		(bioassay 6)	(bioassay 8)
Control with virus (AgMNPV)		80.11±6.45a	84.89±5.67ab
Herb	Bentazone	96.62±1.83a	97.93±0.84a
	Clethodim (Select)	95.14±4.05a	88.26±4.63ab
	Glyphosate-isopropylamine salt	97.19±1.33a	98.62±0.84a
Fung	Azoxystrobin + benzovindiflupyr	80.60±2.87a	69.27±5.30b
	Azoxystrobin + cyproconazole	97.19±2.02a	87.20±2.60ab
	Metaconazole + pyraclostrobin	92.46±5.81a	93.49±1.80a
	Trifloxystrobin + cyproconazole	95.71±3.46a	88.45±2.50ab
Control without virus (Distilled water)		6.67±2.11b	2.00±1.33c
CV (%)		14.63	12.45
F		24.35	44.03
P		<0.0001	<0.0001
$df_{residue}$		36	36

Means ± EPM followed by the same letter in each column do not differ by Tukey's test (5% probability).

and herbicides, allowing its concomitant use, maintaining the control efficiency of *A. gemmatalis*, with mortality rates above 89%. Similar compatible association of pesticides with biological control had been previously published with Roundup Ready® and the parasitoid *Palmistichus elaeisis* Delvare & LaSalle, 1993 (Hymenoptera: Eulophidae) (DE LA CRUZ et al., 2017). Differently, the mixture of oxyfluorfen, glufosinate-ammonium, metribuzin and linuron impaired the entomopathogenic nematodes Rhabditida: Steinernematidae and Heterorhabditidae (LAZNIK & TRDAN, 2017). Fungicides also showed different results depending on the tested products and biological control. Azoxystrobin, chlorothalonil and thi-ophanate-methyl reduced the growth of *Beauveria bassiana* and *Metarhizium anisopliae* (FIEDLER & SOSNOWSKA, 2017). However, for *Orius insidiosus* Say, 1832 (Hemiptera: Anthocoridae), myclobutanil, potassium bicarbonate and cyprodinil + fludioxonil were harmless (GRADISH et al., 2011). In addition to the tank mix of synthetic and biological products, the mix also occurs in seed treatments. The treatment of soybean seed with *Bradyrhizobium* spp mixed with fungicides pyraclostrobin and thiophanate-methyl and the insecticide fipronil, caused a decrease in the efficiency of the bacteria immediately and over time (RODRIGUES et al., 2020). The use of *Thichoderma* spp combined with the fungicide Fludioxonil to treat soybean seed, caused an increase in crop production (ZANDONÁ et al., 2019). Those differences recorded for different biological control agents and chemicals studied illustrate the importance of studying each scenario in order to offer growers the most precise information about what could and could not be mixture.

Only the fungicide (metconazole + pyraclostrobin), caused 40% mortality for *A. gemmatalis,* the effect is possibly linked to the metconazole molecule, which fits into chiral pesticides, in this class the way of action to other organisms is not yet elucidated, in addition 40% of insecticides belong to the chiral class (BIELSKÁ et al., 2021; JESCHKE, 2018). The fungicide (azoxystrobin + benzovindiflupyr) caused a reduction in mortality of *A. gemmatalis* after two hours of mixing (69.27%), compared to one hour of mixing (80.60%), it is possible to infer that this reduction is linked to the increase in pH caused by presence of the fungicide in suspension (LANDIM et al., 2019). Thus, exceeding optimal conditions for virus action. In addition, the substances present in the fungicide formulation, such as surfactants or adjuvants, which have detergent properties and with the longest exposure time, may have caused a deleterious action of the entomopathogen. Molecules with detergent action have hydrophilic and lipophilic portions in their structure, which increases the solubility of the products in water, as well as can denature proteins, which can interfere in the interaction with the virus, reducing the mortality of caterpillars (RAFIKOVA et al., 2004; PETTER et al., 2012). Impact of differences regarding formulation on pesticide selectivity had also been previously reported in the literature for macro-organisms (GIOLO et al., 2006).

The compatibility of AgMNPV with herbicides and fungicides has been proven to occur immediately after mixing and also after two hours of mixing, maintaining the same efficacy with caterpillar mortality close to or above 80%. Therefore, AgMNPV can be mixed with all pesticides tested under laboratory conditions. These laboratory results are essential indications for developing recommendations for use in the field, mainly assessing the quality of the biological agent and following the current legislation on the practice of mixing in a tank.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

AUTHORS' CONTRIBUTION

All the authors contributed equally on the manuscript.

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